Access DB# 49059

SEARCH REQUEST FORM

Scientific and Technical Information Center

Requester's Full Name: Gailene R. Gabel Examiner #: 76197 Date: 8/14/01
Art Unit: 1641 Phone Number 305-1807 Serial Number: 09/485 005
Mail Box and Bldg/Room Location: 7B15 Results Format Preferred (circle): PAPER DISK E-MAIL
7E12

If mor than one search is submitted, please prioritize searches in order of need.

****

Please provide a detailed statement of the search topic, and describe as specifically as possible the subject matter to be searched. Include the elected species or structures, keywords, synonyms, acronyms, and registry numbers, and combine with the concept or utility of the invention. Define any terms that may have a special meaning. Give examples or relevant citations, authors, etc, if known. Please attach a copy of the cover sheet, pertinent claims, and abstract.

Title of Invention: Method of detecting amyloid like fibrils or protein aggregates

Inventors (please provide full names): Wanker, Erich; Lehrach, Hans; Scherzinger, Eberhardt; Bates, Gillian

Earliest Priority Filing Date: 8/1/97

****For Sequence Searches Only* Please include all pertinent information (parent, child, divisional, or issued patent numbers) along with the appropriate serial number.***

Please search highlighted terms on ~~pgs~~
claims 1, 4-7, 10-12, 17, 20, + 24.
and neurodegenerative disease
amyloid?
protein (fusion)

1524
1508-18

Abstract attached

Point of Contact:
Mary Hale
Technical Info. Specialist
CM1 12D16 Tel: 308-4258

Point of Contact:
Mary Hale
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CM1 12D16 Tel: 308-4258

detergent
SDS
Triton X-100

amyloid fibril protein
amyloid beta-protein
" A4 protein precursor
" beta-amyloid protein "
Alzheimers disease

Thanks,
Gail

****

STAFF USE ONLY	Type of Search		Vendors and cost where applicable
Searcher: Mary	NA Sequence (#)		STN 343.94
Searcher Phone #:	AA Sequence (#)		Dialog
Searcher Location:	Structure (#)		Questel/Orbit
Date Searcher Picked Up:	Bibliographic	✓	Dr.Link
Date Completed: 8/17	Litigation		Lexis/Nexis
Searcher Prep & Review Time:	Fulltext		Sequence Systems
Clerical Prep Time:	Patent Family		WWW/Internet
Online Time: 1.6	Other		Other (specify)

PTO-1590 (1-2000)

Gabel
285005

```
=> fil caplu;e urea insoluble amyloid like fibril/ct 5
COST IN U.S. DOLLARS                                SINCE FILE      TOTAL
                                                       ENTRY    SESSION
FULL ESTIMATED COST                                   113.25     113.70

DISCOUNT AMOUNTS (FOR QUALIFYING ACCOUNTS)          SINCE FILE      TOTAL
                                                       ENTRY    SESSION
CA SUBSCRIBER PRICE                                    -4.12      -4.12

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FILE COVERS 1947 - 17 Aug 2001  VOL 135 ISS 9
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```


E#	FREQUENCY	AT	TERM
E1	0	2	UREA FERTILIZERS, CONTROLLED-RELEASE/CT
E2	0	15	UREA HYDROGEN PEROXIDE/CT
E3	0	-->	UREA INSOLUBLE AMYLOID LIKE FIBRIL/CT
E4	0	2	UREA MICROBIAL GENE/CT
E5	0	2	UREA PERMEASE/CT

```
=> e amyloid like fibril/ct 5
E#   FREQUENCY    AT     TERM
--   ---------    --     ----
E1           0     2     AMYLOID ANGIOPATHY BRAIN/CT
E2           0     2     AMYLOID LIGHT-CHAIN IGS/CT
E3           0       --> AMYLOID LIKE FIBRIL/CT
E4           0     2     AMYLOID NEUROPATHY/CT
E5           0     2     AMYLOID POLYNEUROPATHY/CT

=> e protein aggregate/ct 5
E#   FREQUENCY    AT     TERM
--   ---------    --     ----
E1           0     2     PROTEIN A (L) IMMOBILIZED/CT
E2         283     2     PROTEIN ADSORPTION/CT
E3           0       --> PROTEIN AGGREGATE/CT
E4          27     2     PROTEIN BODY/CT
E5           0     2     PROTEIN BODY ORGANELLE/CT

=> e fibril/ct 5
E#   FREQUENCY    AT     TERM
--   ---------    --     ----
E1           2     6     FIBRICOLA SEOULENSIS/CT
E2           0     1     FIBRID/CT
E3         451     4 --> FIBRIL/CT
E4           0     2     FIBRIL (L) ANATOMICAL/CT
E5           0     2     FIBRIL (L) MICRO-/CT

=> e e3+all/ct
E1        8654   BT1  Fibers/CT
E2         451     -->  Fibril/CT
                     HN   Valid heading during volumes 76-125 (1972-1996) and
                          131 (July 1999) to present.
E3           5       OLD  Fibrils/CT
E4          97       OLD  Fibrils (fibers)/CT
*********  END***

=> s e2-4
           451 FIBRIL/CT
             5 FIBRILS/CT
            97 "FIBRILS (FIBERS)"/CT
L1         553 (FIBRIL/CT OR FIBRILS/CT OR "FIBRILS (FIBERS)"/CT)

=> e polyglutamine expansion/ct 5
E#   FREQUENCY    AT     TERM
--   ---------    --     ----
E1           0     2     POLYGLUTAMIC ACID/CT
E2           0     2     POLYGLUTAMIC ACID, SRU/CT
E3           0       --> POLYGLUTAMINE EXPANSION/CT
E4           0     1     POLYGLUTARIMIDE-/CT
E5           0     1     POLYGLUTARIMIDE-POLYESTER-/CT

=> fil reg
COST IN U.S. DOLLARS                          SINCE FILE      TOTAL
                                                 ENTRY    SESSION
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FULL ESTIMATED COST                                    5.31      119.01

DISCOUNT AMOUNTS (FOR QUALIFYING ACCOUNTS)       SINCE FILE       TOTAL
                                                      ENTRY     SESSION
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DICTIONARY FILE UPDATES:  16 AUG 2001  HIGHEST RN 351857-20-0

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  conducting SmartSELECT searches.

Structure search limits have been increased.  See HELP SLIMIT
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=> e polyglutamine/cn 5
E1            1     POLYGLUTAMIC ACID SODIUM SALT, SRU/CN
E2            1     POLYGLUTAMIC ACID, SRU/CN
E3            2 --> POLYGLUTAMINE/CN
E4            1     POLYGLUTAMINE DOMAIN PROTEIN (SACCHAROMYCES CEREVISIAE
GENE
                    PGD1)/CN
E5            1     POLYGLUTAMINE TRACT-BINDING PROTEIN-1 (HUMAN CLONE PQBP-1
GE
                    NE PQBP-1)/CN

=> s e3
L2            2 POLYGLUTAMINE/CN

=> e cellulose acetate/cn 5
E1            1     CELLULOSE 6-PHENYLCARBAMATE
2,3-BIS(3,5-DIMETHYLPHENYLCARBAM
                    ATE)/CN
E2            1     CELLULOSE A/CN
E3            1 --> CELLULOSE ACETATE/CN
E4            1     CELLULOSE ACETATE .EPSILON.-(FORMYLOXY)CAPROATE/CN
E5            1     CELLULOSE ACETATE .EPSILON.-HYDROXYCAPROATE/CN

=> s e3
L3            1 "CELLULOSE ACETATE"/CN

=> e sds/cn 5
E1            1     SDR 1/CN
E2            1     SDR 5175/CN
E3            1 --> SDS/CN
E4            1     SDS 023018/CN
E5            1     SDS 023946/CN
```

```
=> s e3;e triton x100/cn 5
L4            1 SDS/CN


E1            1     TRITON X-800/CN
E2            1     TRITON X-A/CN
E3            0 --> TRITON X100/CN
E4            1     TRITON XL 80N/CN
E5            1     TRITON XN 45S/CN

=> e "triton x-100"/cn 5
E1            1     TRITON X 770/CN
E2            1     TRITON X 800/CN
E3            0 --> TRITON X-100/CN
E4            1     TRITON X-171/CN
E5            1     TRITON X-57/CN

=> fil caplus;e fusion protein/ct 5
COST IN U.S. DOLLARS                                SINCE FILE      TOTAL
                                                       ENTRY    SESSION
FULL ESTIMATED COST                                    12.33     131.34

DISCOUNT AMOUNTS (FOR QUALIFYING ACCOUNTS)          SINCE FILE      TOTAL
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E#    FREQUENCY     AT      TERM
--    ---------     --      ----
E1            0      2      FUSION PRODUCTS, GENE OMPA PORINS/CT
E2            0      2      FUSION PRODUCTS, PROTEIN G/CT
E3            0        --> FUSION PROTEIN/CT
E4            0      3      FUSION PROTEINS/CT
E5         5510     13      FUSION PROTEINS (CHIMERIC PROTEINS)/CT

=> e e5+all/ct
E1          310   BT2  Proteins, general/CT
E2       176456     BT1  Proteins, specific or class/CT
E3         5510       -->  Fusion proteins (chimeric proteins)/CT
                        HN   Valid heading during volume 126 (1997) to present.
E4                      OLD  Proteins (L) chimeric/CT
E5                      OLD  Proteins, specific or class (L) fusion products/CT
E6                      UF   Chimeric proteins/CT
E7                      UF   Fusion products proteins/CT
E8                      UF   Fusion proteins/CT
E9                      UF   Protein, fusion/CT
E10                     UF   Proteins (L) hybrid/CT
E11                     UF   Proteins, fusion/CT
E12                     UF   Proteins, specific or class (L) chimeric/CT
E13       25467         RT   Recombination, genetic/CT
*********  END***

=> s e2-13
        176456 "PROTEINS, SPECIFIC OR CLASS"/CT
          5510 "FUSION PROTEINS (CHIMERIC PROTEINS)"/CT
        455178 PROTEINS/CT
         12998 CHIMERIC/IT
             5 CHIMERICS/IT
         12998 CHIMERIC/IT
                 ((CHIMERIC OR CHIMERICS)/IT)
           744 "PROTEINS (L) CHIMERIC"/CT
        176456 "PROTEINS, SPECIFIC OR CLASS"/CT
        110576 FUSION/IT
           748 FUSIONS/IT
        110888 FUSION/IT
                 ((FUSION OR FUSIONS)/IT)
        301484 PRODUCTS/IT
          2490 "PROTEINS, SPECIFIC OR CLASS (L) FUSION PRODUCTS"/CT
             0 "CHIMERIC PROTEINS"/CT
             0 "FUSION PRODUCTS PROTEINS"/CT
             0 "FUSION PROTEINS"/CT
             0 "PROTEIN, FUSION"/CT
        455178 PROTEINS/CT
         23086 HYBRID/IT
          5383 HYBRIDS/IT
```

```
         26539 HYBRID/IT
                 ((HYBRID OR HYBRIDS)/IT)
          1084 "PROTEINS (L) HYBRID"/CT
             0 "PROTEINS, FUSION"/CT
        176456 "PROTEINS, SPECIFIC OR CLASS"/CT
<------User Break------>
u
=>
=> s e3-13
          5510 "FUSION PROTEINS (CHIMERIC PROTEINS)"/CT
        455178 PROTEINS/CT
         12998 CHIMERIC/IT
             5 CHIMERICS/IT
         12998 CHIMERIC/IT
                 ((CHIMERIC OR CHIMERICS)/IT)
           744 "PROTEINS (L) CHIMERIC"/CT
        176456 "PROTEINS, SPECIFIC OR CLASS"/CT
        110576 FUSION/IT
           748 FUSIONS/IT
        110888 FUSION/IT
                 ((FUSION OR FUSIONS)/IT)
        301484 PRODUCTS/IT
          2490 "PROTEINS, SPECIFIC OR CLASS (L) FUSION PRODUCTS"/CT
             0 "CHIMERIC PROTEINS"/CT
             0 "FUSION PRODUCTS PROTEINS"/CT
             0 "FUSION PROTEINS"/CT
             0 "PROTEIN, FUSION"/CT
        455178 PROTEINS/CT
         23086 HYBRID/IT
          5383 HYBRIDS/IT
         26539 HYBRID/IT
                 ((HYBRID OR HYBRIDS)/IT)
          1084 "PROTEINS (L) HYBRID"/CT
             0 "PROTEINS, FUSION"/CT
        176456 "PROTEINS, SPECIFIC OR CLASS"/CT
         12998 CHIMERIC/IT
             5 CHIMERICS/IT
         12998 CHIMERIC/IT
                 ((CHIMERIC OR CHIMERICS)/IT)
           676 "PROTEINS, SPECIFIC OR CLASS (L) CHIMERIC"/CT
         25467 "RECOMBINATION, GENETIC"/CT
L6       34452 ("FUSION PROTEINS (CHIMERIC PROTEINS)"/CT OR "PROTEINS (L)
CHIME
               RIC"/CT OR "PROTEINS, SPECIFIC OR CLASS (L) FUSION
PRODUCTS"/CT
               OR "CHIMERIC PROTEINS"/CT OR "FUSION PRODUCTS PROTEINS"/CT OR
               "FUSION PROTEINS"/CT OR "PROTEIN, FUSION"/CT OR "PROTEINS (L)
               HYBRID"/CT OR "PROTEINS, FUSION"/CT OR "PROTEINS, SPECIFIC OR
               CLASS (L) CHIMERIC"/CT OR "RECOMBINATION, GENETIC"/CT)

=> fil medl,caplus,biosis,embase,wpids,jicst
COST IN U.S. DOLLARS                          SINCE FILE      TOTAL
                                                 ENTRY    SESSION
FULL ESTIMATED COST                              51.61     182.95
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DISCOUNT AMOUNTS (FOR QUALIFYING ACCOUNTS)        SINCE FILE      TOTAL
                                                      ENTRY    SESSION
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=> s (amyloid fibril protein or amyloid beta protein or amyloid a4 protein or
beta amyloid protein)
L7         6403 FILE MEDLINE
L8         2000 FILE CAPLUS
L9         2644 FILE BIOSIS
L10        5058 FILE EMBASE
L11         125 FILE WPIDS
L12         370 FILE JICST-EPLUS

TOTAL FOR ALL FILES
L13       16600 (AMYLOID FIBRIL PROTEIN OR AMYLOID BETA PROTEIN OR AMYLOID A4
                PROTEIN OR BETA AMYLOID PROTEIN)

=> s (l13 or protein aggregate) and (huntington or muscular atrophy or
pallidoluysian atrophy or ataxia or alzheimer or amyloidosis or diabetes or
encephalo? or kuru or gerstmann or straussler scheinker syndrome or gss or
insomnia or parkinson)
L14        5342 FILE MEDLINE
L15        1780 FILE CAPLUS
L16        2240 FILE BIOSIS
L17        4498 FILE EMBASE
L18         125 FILE WPIDS
L19         319 FILE JICST-EPLUS

TOTAL FOR ALL FILES
L20       14304 (L13 OR PROTEIN AGGREGATE) AND (HUNTINGTON OR MUSCULAR ATROPHY
                OR PALLIDOLUYSIAN ATROPHY OR ATAXIA OR ALZHEIMER OR
AMYLOIDOSIS
                OR DIABETES OR ENCEPHALO? OR KURU OR GERSTMANN OR STRAUSSLER
                SCHEINKER SYNDROME OR GSS OR INSOMNIA OR PARKINSON)

=> s l20 and filter and (low protein adsorp? or cellulose acetate or l3)
```

L21 0 FILE MEDLINE
L22 1 FILE CAPLUS
L23 0 FILE BIOSIS
L24 0 FILE EMBASE
L25 0 FILE WPIDS
L26 0 FILE JICST-EPLUS

TOTAL FOR ALL FILES
L27 1 L20 AND FILTER AND (LOW PROTEIN ADSORP? OR CELLULOSE ACETATE OR
L3)

=> d cbib abs;s l20 and (l4 or sds or triton x 100)

L27 ANSWER 1 OF 1 CAPLUS COPYRIGHT 2001 ACS
1999:113882 Document No. 130:193967 Novel method of detecting amyloid-like fibrils or **protein aggregates** using **filters** for disease diagnosis and inhibitor identification. Wanker, Erich; Lehrach, Hans; Scherzinger, Eberhard; Bates, Gillian (Max-Planck-Gesellschaft zur Forderung der Wissenschaften e.V., Germany). PCT Int. Appl. WO 9906838 A2 19990211, 56 pp. DESIGNATED STATES: W: CA, JP, US; RW: AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE. (English). CODEN: PIXXD2. APPLICATION: WO 1998-EP4810 19980731.
AB The present invention relates to methods of detecting the presence of detergent- or urea-insol. amyloid-like fibrils or **protein aggregates** on **filters**. Preferably, the fibrils or aggregates are indicative of a disease, preferably of a neurodegenerative disease such as **Alzheimer**'s disease or **Huntington**'s disease. In addn., the present invention relates to inhibitors identified by the method of the invention, to pharmaceutical compns. comprising the inhibitors and to diagnostic compns. useful for the investigation of the amyloid-like fibrils or aggregates. Protein samples were treated with SDS and filtered through **cellulose acetate** membranes in a BRL dot blot filtration unit. The **filters** were washed with SDS soln., blocked, treated with antibody, labeled with secondary antibody-peroxidase conjugate or other detection system, and quantified.

L28 104 FILE MEDLINE
L29 52 FILE CAPLUS
L30 41 FILE BIOSIS
L31 90 FILE EMBASE
L32 2 FILE WPIDS
L33 5 FILE JICST-EPLUS

TOTAL FOR ALL FILES
L34 294 L20 AND (L4 OR SDS OR TRITON X 100)

=> s l34 and (l6 or fusion protein) and (diagno? or detect? or method)
L35 3 FILE MEDLINE
L36 1 FILE CAPLUS
L37 1 FILE BIOSIS

L38 0 FILE EMBASE
L39 0 FILE WPIDS
L40 0 FILE JICST-EPLUS

TOTAL FOR ALL FILES
L41 5 L34 AND (L6 OR FUSION PROTEIN) AND (DIAGNO? OR DETECT? OR METHOD
)

=> s l41 not l27
L42 3 FILE MEDLINE
L43 0 FILE CAPLUS
L44 1 FILE BIOSIS
L45 0 FILE EMBASE
L46 0 FILE WPIDS
L47 0 FILE JICST-EPLUS

TOTAL FOR ALL FILES
L48 4 L41 NOT L27

=> dup rem l48
PROCESSING COMPLETED FOR L48
L49 4 DUP REM L48 (0 DUPLICATES REMOVED)

=> d 1-4 cbib abs

L49 ANSWER 1 OF 4 MEDLINE
2000283963 Document Number: 20283963. PubMed ID: 10801983. Presenilin 1 is linked with gamma-secretase activity in the detergent solubilized state. Li Y M; Lai M T; Xu M; Huang Q; DiMuzio-Mower J; Sardana M K; Shi X
P; Yin K C; Shafer J A; Gardell S J. (Department of Biological Chemistry, Merck Research Laboratories, West Point, PA 19486, USA.. yueming_li@merck.com) . PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF
THE UNITED STATES OF AMERICA, (2000 May 23) 97 (11) 6138-43. Journal code: PV3; 7505876. ISSN: 0027-8424. Pub. country: United States. Language: English.
AB gamma-Secretase is a membrane-associated protease that cleaves within the transmembrane region of amyloid precursor protein to generate the C termini of the two Abeta peptide isoforms, Abeta40 and Abeta42. Here we report the detergent solubilization and partial characterization of gamma-secretase. The activity of solubilized gamma-secretase was measured with a recombinant substrate, C100Flag, consisting largely of the C-terminal fragment of amyloid precursor protein downstream of the beta-secretase cleavage site. Cleavage of C100Flag by gamma-secretase was **detected** by electrochemiluminescence using antibodies that specifically recognize the Abeta40 or Abeta42 termini. Incubation of C100Flag with HeLa cell membranes or detergent-solubilized HeLa cell membranes generates both the Abeta40 and Abeta42 termini. Recovery of catalytically competent, soluble gamma-secretase critically depends on the
choice of detergent; CHAPSO (3-[(3-cholamidopropyl)dimethylammonio]-2-hydroxy-1-propanesulfonate) but not **Triton X-100** is suitable. Solubilized gamma-secretase activity is inhibited

by pepstatin and more potently by a novel aspartyl protease transition-state analog inhibitor that blocks formation of Abeta40 and Abeta42 in mammalian cells. Upon gel exclusion chromatography, solubilized gamma-secretase activity coelutes with presenilin 1 (PS1) at an apparent relative molecular weight of approximately 2.0 x 10(6). Anti-PS1 antibody immunoprecipitates gamma-secretase activity from the solubilized gamma-secretase preparation. These data suggest that gamma-secretase activity is catalyzed by a PS1-containing macromolecular complex.

L49 ANSWER 2 OF 4 MEDLINE

2000391714 Document Number: 20294902. PubMed ID: 10833395. Functional human insulin-degrading enzyme can be expressed in bacteria. Chesneau V; Rosner M R. (Ben May Institute for Cancer Research, University of Chicago, Illinois 60637, USA.) PROTEIN EXPRESSION AND PURIFICATION, (2000 Jun) 19 (1) 91-8. Journal code: BJV; 9101496. ISSN: 1046-5928. Pub. country: United States. Language: English.

AB Insulin-degrading enzyme (IDE) has been shown to degrade a number of biologically important peptides, including insulin and the **amyloid -beta protein** implicated in **Alzheimer's** disease. However, lack of a facile **method** to generate purified enzyme and related mutants has made it difficult to study the precise role of IDE in the clearance of these peptides. Therefore, we determined whether recombinant wild-type and mutant human IDEs can be overexpressed as functional enzymes in bacteria. Three vectors carrying cDNAs encoding N-terminally polyhistidine-tagged recombinant IDEs were constructed, and the proteins expressed in Escherichia coli were purified by metal affinity chromatography (final yield approximately 8 mg per liter of culture). The recombinant IDEs, like the endogenous mammalian enzyme, migrate with 110-kDa apparent molecular masses in **SDS**-polyacrylamide gels and as a approximately 200-kDa species in gel filtration. Further analysis by native PAGE indicates that IDE can form multimers of different complexities. The wild-type recombinant endopeptidase degrades insulin with an efficiency similar to that of the enzyme purified from mammalian tissues. Purified IDEs are stable at 4 degrees C for at least 1 month. Purified recombinant protein was used to raise specific polyclonal antibodies that can immunoprecipitate native mammalian IDE. Thus, the procedure described allows the rapid production of large amounts of purified IDE and demonstrates that IDE can be produced in an active form in the absence of other potential interacting mammalian proteins. Copyright 2000 Academic Press.

L49 ANSWER 3 OF 4 BIOSIS COPYRIGHT 2001 BIOSIS

1999:118696 Document No.: PREV199900118696. Polyglutamine residues from Machado-Joseph disease gene enhance formation of aggregates of GST-polyglutamine **fusion protein** in E. coli. Rhim, Hyangshuk; Bok, Kyoung-Sook; Chang, Mi-Jeong; Kim, In-Kyung; Park, Sung Sup; Kang, Seongman (1). (1) Graduate Sch. Biotechnology, Korea Univ., Seoul 136-701 South Korea . Journal of Microbiology and Biotechnology, (Dec., 1998) Vol. 8, No. 6, pp. 663-668. ISSN: 1017-7825. Language: English.

AB Several neurodegenerative diseases such as **Huntington's** disease,

dentatorubralpallidoluysian atrophy, spinobulbar **muscular atrophy**, Machado-Joseph disease, and spinocerebellar **ataxias** type 1 are associated with the aggregation of expanded glutamine repeats within their proteins. Generally, in clinically affected individuals, the expansion of the polyglutamine sequences is beyond 40 residues. To address the length of polyglutamine that forms aggregation, we have constructed plasmids encoding glutathione S-transferase (GST) Machado-Joseph disease gene **fusion proteins** containing polyglutamine and investigated the formation of aggregates in E. coli. Surprisingly, even (Gln)8 in the normal range as well as (Gln)65 in the pathogenic range enhanced the formation of insoluble **protein aggregates**, whereas (Ser)8 and (Ala)8 did not form aggregates. Our results indicate that the formation of **protein aggregates** in GST-polyglutamine proteins is specifically mediated by the polyglutamine repeat sequence within their protein structure. Our study may contribute to the understanding of the molecular mechanism of the formation of **protein aggregates** in neurodegenerative disorders and the development of preventative strategies.

L49 ANSWER 4 OF 4 MEDLINE

97344255 Document Number: 97344255. PubMed ID: 9224643. Apolipoprotein E forms stable complexes with recombinant **Alzheimer**'s disease beta-amyloid precursor protein. Haas C; Cazorla P; Miguel C D; Valdivieso F; Vazquez J. (Centro de Biologia Molecular 'Severo Ochoa', Universidad Autonoma de Madrid, 28049 Madrid, Spain.) BIOCHEMICAL JOURNAL, (1997 Jul 1) 325 (Pt 1) 169-75. Journal code: 9YO; 2984726R. ISSN: 0264-6021. Pub. country: ENGLAND: United Kingdom. Language: English.

AB Apolipoprotein E (apoE), a protein genetically linked to the incidence of **Alzheimer**'s disease, forms **SDS**-stable complexes in vitro with beta-amyloid peptide (Abeta), the primary component of senile plaques. In the present study, we investigated whether apoE was able to bind full-length Abeta precursor protein (APP). Using a maltose-binding-protein-APP **fusion protein** and human very-low-density lipoprotein (VLDL), we **detected** an interaction of apoE with APP that was inhibited by Abeta or anti-apoE antibody. Saturation-binding experiments indicated a single binding equilibrium with an apparent 1:1 stoichiometry and a dissociation constant of 15 nM. An interaction was also observed using apoE from cerebrospinal fluid or delipidated VLDL, as well as recombinant apoE. APP.apoE complexes were **SDS**-stable, and their formation was not inhibited by reducing conditions; however, they were dissociated by **SDS** under reducing conditions. ApoE.APP complexes formed high-molecular-mass aggregates, and competition experiments suggested that amino acids 14-23 of Abeta are responsible for complex-formation. Finally, no differences were found when studying the interaction of APP with apoE3 or apoE4. Taken together, our results demonstrate that apoE may form stable complexes with the Abeta moiety of APP with characteristics similar to those of complexes formed with isolated Abeta, and suggest the intriguing possibility that apoE-APP interactions may be pathologically relevant in vivo.

```
=> s (l2 or polyglutamine)(4a)expans? and l34
L50          0 FILE MEDLINE
L51          1 FILE CAPLUS
L52          1 FILE BIOSIS
L53          0 FILE EMBASE
L54          0 FILE WPIDS
L55          0 FILE JICST-EPLUS

TOTAL FOR ALL FILES
L56          2 (L2 OR POLYGLUTAMINE)(4A) EXPANS? AND L34

=> dup rem l56
PROCESSING COMPLETED FOR L56
L57           2 DUP REM L56 (0 DUPLICATES REMOVED)

=> d cbib abs 1-2

L57  ANSWER 1 OF 2  CAPLUS  COPYRIGHT 2001 ACS
1999:113882  Document No. 130:193967  Novel method of detecting amyloid-like
     fibrils or protein aggregates using filters for
     disease diagnosis and inhibitor identification.  Wanker, Erich; Lehrach,
     Hans; Scherzinger, Eberhard; Bates, Gillian (Max-Planck-Gesellschaft zur
     Forderung der Wissenschaften e.V., Germany).  PCT Int. Appl. WO 9906838
A2
     19990211, 56 pp.  DESIGNATED STATES: W:  CA, JP, US; RW: AT, BE, CH, CY,
     DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE.  (English).
     CODEN: PIXXD2.  APPLICATION: WO 1998-EP4810 19980731.
AB   The present invention relates to methods of detecting the presence of
     detergent- or urea-insol. amyloid-like fibrils or protein
     aggregates on filters.  Preferably, the fibrils or aggregates are
     indicative of a disease, preferably of a neurodegenerative disease such
as
     Alzheimer's disease or Huntington's disease.  In addn.,
     the present invention relates to inhibitors identified by the method of
     the invention, to pharmaceutical compns. comprising the inhibitors and to
     diagnostic compns. useful for the investigation of the amyloid-like
     fibrils or aggregates.  Protein samples were treated with SDS
     and filtered through cellulose acetate membranes in a BRL dot blot
     filtration unit.  The filters were washed with SDS soln.,
     blocked, treated with antibody, labeled with secondary
antibody-peroxidase
     conjugate or other detection system, and quantified.

L57  ANSWER 2 OF 2  BIOSIS  COPYRIGHT 2001 BIOSIS
1999:118696  Document No.: PREV199900118696. Polyglutamine residues from
     Machado-Joseph disease gene enhance formation of aggregates of
     GST-polyglutamine fusion protein in E. coli. Rhim, Hyangshuk; Bok,
     Kyoung-Sook; Chang, Mi-Jeong; Kim, In-Kyung; Park, Sung Sup; Kang,
     Seongman (1). (1) Graduate Sch. Biotechnology, Korea Univ., Seoul 136-701
     South Korea                                . Journal of Microbiology and
     Biotechnology, (Dec., 1998) Vol. 8, No. 6, pp. 663-668. ISSN: 1017-7825.
     Language: English.
AB   Several neurodegenerative diseases such as Huntington's disease,
     dentatorubralpallidoluysian atrophy, spinobulbar muscular
```

atrophy, Machado-Joseph disease, and spinocerebellar **ataxias** type 1 are associated with the aggregation of expanded glutamine repeats within their proteins. Generally, in clinically affected individuals, the **expansion** of the **polyglutamine** sequences is beyond 40 residues. To address the length of polyglutamine that forms aggregation, we have constructed plasmids encoding glutathione S-transferase (GST) Machado-Joseph disease gene fusion proteins containing polyglutamine and investigated the formation of aggregates in E. coli. Surprisingly, even (Gln)8 in the normal range as well as (Gln)65 in the pathogenic range enhanced the formation of insoluble **protein aggregates**, whereas (Ser)8 and (Ala)8 did not form aggregates. Our results indicate that the formation of **protein aggregates** in GST-polyglutamine proteins is specifically mediated by the polyglutamine repeat sequence within their protein structure. Our study may contribute to the understanding of the molecular mechanism of the formation of **protein aggregates** in neurodegenerative disorders and the development of preventative strategies.

```
=> s l34 and (microscop? or fluorescen? or chemiluminescen?) and (antibod? or
polypeptide or tag or enzyme or assay?)
L58          15 FILE MEDLINE
L59           2 FILE CAPLUS
L60           2 FILE BIOSIS
L61           9 FILE EMBASE
L62           1 FILE WPIDS
L63           0 FILE JICST-EPLUS

TOTAL FOR ALL FILES
L64          29 L34 AND (MICROSCOP? OR FLUORESCEN? OR CHEMILUMINESCEN?) AND
                (ANTIBOD? OR POLYPEPTIDE OR TAG OR ENZYME OR ASSAY?)

=> s l64 and (tissue or cell or bacteria or yeast or fungi or fungus or plant
or insect or animal or mammal or transgen?)
L65          11 FILE MEDLINE
L66           2 FILE CAPLUS
L67           1 FILE BIOSIS
L68           8 FILE EMBASE
L69           1 FILE WPIDS
L70           0 FILE JICST-EPLUS

TOTAL FOR ALL FILES
L71          23 L64 AND (TISSUE OR CELL OR BACTERIA OR YEAST OR FUNGI OR
FUNGUS
                OR PLANT OR INSECT OR ANIMAL OR MAMMAL OR TRANSGEN?)

=> s wanker e?/au,in;s lehrach h?/au,in;s scherzinger e?/au,in
'IN' IS NOT A VALID FIELD CODE
L72          28 FILE MEDLINE
L73          36 FILE CAPLUS
L74          32 FILE BIOSIS
'IN' IS NOT A VALID FIELD CODE
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L75          26 FILE EMBASE
L76           6 FILE WPIDS
L77           1 FILE JICST-EPLUS

TOTAL FOR ALL FILES
L78         129 WANKER E?/AU,IN


'IN' IS NOT A VALID FIELD CODE
L79         301 FILE MEDLINE
L80         362 FILE CAPLUS
L81         417 FILE BIOSIS
'IN' IS NOT A VALID FIELD CODE
L82         253 FILE EMBASE
L83          15 FILE WPIDS
L84           0 FILE JICST-EPLUS

TOTAL FOR ALL FILES
L85        1348 LEHRACH H?/AU,IN


'IN' IS NOT A VALID FIELD CODE
L86          45 FILE MEDLINE
L87          49 FILE CAPLUS
L88          44 FILE BIOSIS
'IN' IS NOT A VALID FIELD CODE
L89          31 FILE EMBASE
L90           3 FILE WPIDS
L91           0 FILE JICST-EPLUS

TOTAL FOR ALL FILES
L92         172 SCHERZINGER E?/AU,IN

=> s l71 not (l56 or l41 or l27);s l92 and l85 and 78
L93          11 FILE MEDLINE
L94           1 FILE CAPLUS
L95           1 FILE BIOSIS
L96           8 FILE EMBASE
L97           1 FILE WPIDS
L98           0 FILE JICST-EPLUS

TOTAL FOR ALL FILES
L99          22 L71 NOT (L56 OR L41 OR L27)


L100          0 FILE MEDLINE
L101          1 FILE CAPLUS
L102          0 FILE BIOSIS
L103          0 FILE EMBASE
L104          0 FILE WPIDS
L105          0 FILE JICST-EPLUS

TOTAL FOR ALL FILES
L106          1 L92 AND L85 AND 78
```

=> dup rem l99
PROCESSING COMPLETED FOR L99
L107 15 DUP REM L99 (7 DUPLICATES REMOVED)

=> d 1-15 cbib abs hit;d l106 cbib abs

L107 ANSWER 1 OF 15 WPIDS COPYRIGHT 2001 DERWENT INFORMATION LTD
AN 2001-191517 [19] WPIDS
AB WO 200110900 A UPAB: 20010405
NOVELTY - An isolated soluble non-fibrillar amyloid beta oligomeric structure (I), comprising 3-24 **amyloid beta proteins** that does not contain an exogenous added crosslinking agent, and which exhibits neurotoxic activity, is new.
DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following:
(1) **assaying** (II) the effects of (I), comprising:
(a) administering (I) to the hippocampus of an **animal**;
(b) applying an electrical stimulus; and
(c) measuring the **cell** body spike amplitude over time to determine the long-term potentiation response;
(2) protecting an **animal** against or reversing decreases in learning or memory due to the effects of (I), by administering a compound that blocks the formation or activity of (I);
(3) protecting a nerve **cell** against or reversing decreases in long-term potentiation or aberrant neuronal signaling due to the effects of (I), by contacting the **cell** with a compound that blocks the formation or activity of (I);
(4) detecting (I) in a test material (TM), comprising:
(a) contacting TM with 6E10 **antibody** and detecting binding of **antibody** to (I);
(b) contacting TM with serum-starved neuroblastoma **cells**, and measuring morphological changes in the **cells** by comparing the morphology of **cells** against neuroblastoma **cells** that have not been contacted with TM;
(c) contacting TM with brain slice cultures, and measuring brain **cell** death compared to brain slice cultures that have not been contacted with TM;
(d) contacting TM with neuroblastoma **cells** and measuring increases in Fyn kinase activity by comparing Fyn kinase activity in **cells** against Fyn kinase activity in neuroblastoma **cells** that have not been contacted with TM;
(e) contacting TM with cultures of primary astrocytes, and determining activation of astrocytes compared to cultures of primary astrocytes not contacted with TM, or measuring in the astrocytes increases in the mRNA for proteins such as interleukin-1, inducible nitric oxide synthase, Apo E, Apo J, and alpha 1-antichymotrypsin by comparing mRNA levels in the astrocytes against the corresponding mRNA levels in cultures of primary astrocytes not contacted with TM; or
(f) contacting TM with a nerve **cell** and determining if the **cell** exhibits amyloid beta-derived dementing ligands (ADDL)-induced aberrant neuronal signaling;
(5) identifying (III) compounds that modulate the effects of (I), by:

(a) administering either saline or a test compound to the hippocampus of an **animal**;

(b) applying an electric stimulus;

(c) measuring the **cell** body spike amplitude over time to determine the long-term potentiation response; and

(d) comparing the long-term potentiation response of **animals** having saline administered to the long-term potentiation of **animals** having test compounds administered, with the proviso that administration of (I) is not done by therapy;

(6) identifying compounds that block the neurotoxicity of (I), by:

(a) contacting separate cultures of neuronal **cells** with (I) either in the presence or absence of contacting with test compound;

(b) measuring the proportion of viable **cells** in each culture; and

(c) comparing the proportion with compounds that block the neurotoxicity of (I) being identified as resulting in an increased proportion of viable **cells** in culture as compared to the corresponding culture contacted with (I) in the absence of test compound;

(7) identifying compounds that block binding to a **cell** surface protein of (I), comprising:

(a) forming (I) from **amyloid beta - protein** so that it becomes a labeled oligomeric structure comprising a binding moiety capable of binding **fluorescent** reagent;

(b) contacting separate cultures of neuronal **cells** with labeled (I) either in the presence or absence of contacting with test compound;

(c) adding a **fluorescent** reagent that binds to (I);

(d) analyzing separate **cell** cultures by **fluorescence**-activated **cell** sorting;

(e) comparing the fluorescence of the cultures, with compounds that block binding to a cell surface protein of (I) being identified as resulting in a reduced fluorescence of culture as compared to the corresponding culture contacted with (I) in the absence of test compound;

(8) identifying (IV) compounds that block formation or binding to a cell surface protein of (I), by preparing separate samples of amyloid beta protein that have or have not been mixed with test compound, forming (I) in separate samples, where optionally (I) becomes labeled comprising a binding moiety capable of binding a fluorescent reagent in each separate samples and performing steps (b)-(e) of (7);

(9) detecting (V) binding to a cell surface protein of (I) or identifying compounds that block the binding, comprising:

(a) forming (I) form amyloid beta protein;

(b) contacting a culture of neuronal cells with (I);

(c) adding an antibody including a conjugating moiety that binds (I) and washing away unbound antibody;

(d) linking an enzyme to antibody bound to (I) by conjugating moiety;

(e) adding a colorless substrate that is cleaved by enzyme to yield a color change; and

(f) determining color change as a measure of binding to a cell surface protein of (I) or comparing the color change produced by each of

the separate samples, with compounds that block formation or binding to a cell surface protein of (I) being identified as resulting in a reduced color change produced by the culture as compared to the corresponding culture contacted with (I) in the absence of test compound; and

(10) preparation (VI) of (I), by:

(a) obtaining a solution of monomeric amyloid beta protein, diluting protein solution to a final concentration of 5 nM-500 micro M, incubating the solution at 4 deg. C for 2-48 hours, centrifuging to 14000 g at 4 deg.

C, and recovering the supernatant as containing (I); or

(b) obtaining a solution of monomeric amyloid beta protein, dissolving the monomer in hexafluoroisopropanol, removing hexafluoroisopropanol by speed vacuum evaporation to obtain solid peptide,

dissolving the peptide in dimethylsulfoxide (DMSO) to form a DMSO stock solution, diluting the solution, vortexing and incubating at 4 deg. C for 24 hours.

ACTIVITY - Cytostatic; nootropic; neuroprotective; vulnerary.

No biological data is given.

MECHANISM OF ACTION - ADDL-modulator.

USE - (III) and (IV) are useful for identifying compounds that increase or decrease the formation and/or activity of (I). The compounds are useful in the treatment of diseases, disorders or conditions due to the effect of (I), that manifests as a deficit in cognition, learning and/or memory, especially Alzheimer's disease, adult Down's syndrome and senile dementia. (I) is itself useful in activating endothelial cells for treating wound healing and to selectively destroy targeted neural cells e.g. in cases of cancer, in particular brain cancer.

Dwg.0/17

TI Amyloid beta-derived dementing ligands for treating cancer, whose modulators are useful in treating learning, memory disorders, has **amyloid beta-protein** assembled into globular non-fibrillar oligomeric structures.

AB WO 200110900 A UPAB: 20010405

NOVELTY - An isolated soluble non-fibrillar amyloid beta oligomeric structure (I), comprising 3-24 **amyloid beta proteins** that does not contain an exogenous added crosslinking agent, and which exhibits neurotoxic activity, is new.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following:

(1) **assaying** (II) the effects of (I), comprising:

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(b) applying an electrical stimulus; and

(c) measuring the **cell** body spike amplitude over time to determine the long-term potentiation response;

(2) protecting an **animal** against or reversing decreases in learning or memory due to the effects of (I), by administering a compound that blocks the formation or activity of (I);

(3) protecting a nerve **cell** against or reversing decreases in long-term potentiation or aberrant neuronal signaling due to the effects of (I), by contacting the **cell** with a compound that blocks the formation or activity of (I);

(4) detecting (I) in a test material (TM), comprising:

(a) contacting TM with 6E10 **antibody** and detecting binding of **antibody** to (I);

(b) contacting TM with serum-starved neuroblastoma **cells**, and measuring morphological changes in the **cells** by comparing the morphology of **cells** against neuroblastoma **cells** that have not been contacted with TM;

(c) contacting TM with brain slice cultures, and measuring brain **cell** death compared to brain slice cultures that have not been contacted with TM;

(d) contacting TM with neuroblastoma **cells** and measuring increases in Fyn kinase activity by comparing Fyn kinase activity in **cells** against Fyn kinase activity in neuroblastoma **cells** that have not been contacted with TM;

(e) contacting TM with cultures of primary astrocytes, and determining activation of astrocytes compared to cultures of primary astrocytes not contacted with TM, or measuring in the astrocytes increases in the mRNA for proteins such as interleukin-1, inducible nitric oxide synthase, Apo E, Apo J, and alpha 1-antichymotrypsin by comparing mRNA levels in the astrocytes against the corresponding mRNA levels in cultures of primary astrocytes not contacted with TM; or

(f) contacting TM with a nerve **cell** and determining if the **cell** exhibits amyloid beta-derived dementing ligands (ADDL)-induced aberrant neuronal signaling;

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(b) applying an electric stimulus;

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(a) forming (I) from **amyloid beta - protein** so that it becomes a labeled oligomeric structure comprising a binding moiety capable of binding **fluorescent** reagent;

(b) contacting separate cultures of neuronal **cells** with labeled (I) either in the presence or absence of contacting with test compound;

(c) adding a **fluorescent** reagent that binds to (I);

(d) analyzing separate **cell** cultures by

fluorescence-activated **cell** sorting;

(e) comparing the fluorescence of the cultures, with compounds that block binding to a cell surface protein of (I) being identified as resulting in a reduced fluorescence of culture as compared to the corresponding culture contacted with (I) in the absence of test compound;

(8) identifying (IV) compounds that block formation or binding to a cell surface protein of (I), by preparing separate samples of amyloid beta protein that have or have not been mixed with test compound, forming (I) in separate samples, where optionally (I) becomes labeled comprising a binding moiety capable of binding a fluorescent reagent in each separate samples and performing steps (b)-(e) of (7);

(9) detecting (V) binding to a cell surface protein of (I) or identifying compounds that block the binding, comprising:

(a) forming (I) form amyloid beta protein;

(b) contacting a culture of neuronal cells with (I);

(c) adding an antibody including a conjugating moiety that binds (I) and washing away unbound antibody;

(d) linking an enzyme to antibody bound to (I) by conjugating moiety;

(e) adding a colorless substrate that is cleaved by enzyme to yield a color change; and

(f) determining color change as a measure of binding to a cell surface protein of (I) or comparing the color change produced by each of the separate samples, with compounds that block formation or binding to a cell surface protein of (I) being identified as resulting in a reduced color change produced by the culture as compared to the corresponding culture contacted with (I) in the absence of test compound; and

(10) preparation (VI) of (I), by:

(a) obtaining a solution of monomeric amyloid beta protein, diluting protein solution to a final concentration of 5 nM-500 micro M, incubating the solution at 4 deg. C for 2-48 hours, centrifuging to 14000 g at 4 deg. C, and recovering the supernatant as containing (I); or

(b) obtaining a solution of monomeric amyloid beta protein, dissolving the monomer in hexafluoroisopropanol, removing hexafluoroisopropanol by speed vacuum evaporation to obtain solid peptide, dissolving the peptide in dimethylsulfoxide (DMSO) to form a DMSO stock solution, diluting the solution, vortexing and incubating at 4 deg. C for 24 hours.

ACTIVITY - Cytostatic; nootropic; neuroprotective; vulnerary.

No biological data is given.

MECHANISM OF ACTION - ADDL-modulator.

USE - (III) and (IV) are useful for identifying compounds that increase or decrease the formation and/or activity of (I). The compounds are useful in the treatment of diseases, disorders or conditions due to the effect of (I), that manifests as a deficit in cognition, learning and/or memory, especially Alzheimer's disease, adult Down's syndrome and senile dementia. (I) is itself useful in activating endothelial cells for treating wound healing and to selectively destroy targeted neural cells e.g. in cases of cancer, in particular brain cancer.

Dwg.0/17

TECH UPTX: 20010405

TECHNOLOGY FOCUS - BIOTECHNOLOGY - Preferred Oligomeric Structure: (I) comprises 3-8 mer, 12-mer, 16-mer, 20-mer or 24-mer aggregates of **amyloid beta-proteins**. (I) has a molecular weight of 36-108 kDa, preferably 26-28 kDa, as determined by non-denaturing gel electrophoresis, 22-24 or 18-19 kDa as determined by electrophoresis in 15 % sodium dodecyl sulfate (**SDS**)-polyacrylamide gel or 13-116 kDa as determined by electrophoresis on a 16.5 % tris-tricine **SDS**-polyacrylamide gel. 40-75 % of (I) comprises globules of dimension 6.5-11, 4.9-5.4 or 5.7-6.2 nm as measured by atomic force **microscopy**.
Preferred Method: In (II) the long-term potentiation response of the **animal** is compared to that of another **animal** administered saline instead of (I) prior to application of electrical stimulus. (III) further comprises administering (I) to hippocampus either before or after administering saline or test compound. While preparing (I), step (a) of (VI) comprises incubating the media at 4 degrees C in the presence of clusterin.

TT TT: AMYLOID BETA DERIVATIVE LIGAND TREAT CANCER MODULATE USEFUL TREAT LEARNING MEMORY DISORDER **AMYLOID BETA PROTEIN** ASSEMBLE GLOBULAR NON FIBRIL OLIGOMERISE STRUCTURE.

L107 ANSWER 2 OF 15 MEDLINE DUPLICATE 1
2000300971 Document Number: 20300971. PubMed ID: 10829068. Inhibition of huntingtin fibrillogenesis by specific **antibodies** and small molecules: implications for **Huntington**'s disease therapy. Heiser V; Scherzinger E; Boeddrich A; Nordhoff E; Lurz R; Schugardt N; Lehrach H; Wanker E E. (Max-Planck-Institut fur Molekulare Genetik, Ihnestrassee 73, D-14195 Berlin, Germany.) PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, (2000 Jun 6) 97 (12) 6739-44. Journal code: PV3; 7505876. ISSN: 0027-8424. Pub. country: United States. Language: English.

AB The accumulation of insoluble **protein aggregates** in intra and perinuclear inclusions is a hallmark of **Huntington**'s disease (HD) and related glutamine-repeat disorders. A central question is whether protein aggregation plays a direct role in the pathogenesis of these neurodegenerative diseases. Here we show by using a filter retardation **assay** that the mAb 1C2, which specifically recognizes the elongated polyglutamine (polyQ) stretch in huntingtin, and the chemical compounds Congo red, thioflavine S, chrysamine G, and Direct fast yellow inhibit HD exon 1 protein aggregation in a dose-dependent manner. On the other hand, potential inhibitors of amyloid-beta formation such as thioflavine T, gossypol, melatonin, and rifampicin had little or no inhibitory effect on huntingtin aggregation in vitro. The results obtained by the filtration **assay** were confirmed by electron **microscopy**, **SDS**/PAGE, and MS. Furthermore, **cell** culture studies revealed that the Congo red dye at micromolar concentrations reduced the extent of HD exon 1 aggregation in transiently transfected COS **cells**. Together, these findings contribute to a better understanding of the mechanism of huntingtin fibrillogenesis in vitro and provide the basis for the development of new huntingtin aggregation inhibitors that may be effective in treating HD.

TI Inhibition of huntingtin fibrillogenesis by specific **antibodies** and small molecules: implications for **Huntington**'s disease therapy.

AB The accumulation of insoluble **protein aggregates** in intra and perinuclear inclusions is a hallmark of **Huntington**'s disease (HD) and related glutamine-repeat disorders. A central question is whether protein aggregation plays a direct role in the pathogenesis of these neurodegenerative diseases. Here we show by using a filter retardation **assay** that the mAb 1C2, which specifically recognizes the elongated polyglutamine (polyQ) stretch in huntingtin, and the chemical compounds Congo red, thioflavine S, chrysamine G, and Direct fast yellow inhibit HD exon 1 protein aggregation in a dose-dependent manner. On the other hand, potential inhibitors of amyloid-beta formation such as thioflavine T, gossypol, melatonin, and rifampicin had little or no inhibitory effect on huntingtin aggregation in vitro. The results obtained by the filtration **assay** were confirmed by electron **microscopy**, **SDS**/PAGE, and MS. Furthermore, **cell** culture studies revealed that the Congo red dye at micromolar concentrations reduced the extent of HD exon 1 aggregation in transiently transfected COS **cells**. Together, these findings contribute to a better understanding of the mechanism of huntingtin fibrillogenesis in vitro and provide the basis for the development of new huntingtin aggregation inhibitors that may be effective in treating HD.

CT Check Tags: **Animal**; Human; Support, Non-U.S. Gov't
***Antibodies, Monoclonal: TU, therapeutic use**
Benzoates: PD, pharmacology
Biphenyl Compounds: PD, pharmacology
COS Cells
Congo Red: PD, pharmacology
Gossypol: PD, pharmacology
***Huntington Disease: TH, therapy**
Melatonin: PD, pharmacology
***Nerve Tissue Proteins: AI, antagonists & inhibitors**
*Nuclear Proteins: AI, antagonists & inhibitors
*Peptides: AI, antagonists & inhibitors
Rifampin: PD, pharmacology
Thiazoles: PD, pharmacology

CN 0 (**Antibodies**, Monoclonal); 0 (Benzoates); 0 (Biphenyl Compounds); 0 (Huntingtin protein); 0 (Nerve **Tissue** Proteins); 0 (Nuclear Proteins); 0 (Peptides); 0 (Thiazoles)

L107 ANSWER 3 OF 15 MEDLINE

1999445335 Document Number: 99445335. PubMed ID: 10514400. Non-Abeta component of **Alzheimer**'s disease amyloid (NAC) revisited. NAC and alpha-synuclein are not associated with Abeta amyloid. Culvenor J G; McLean C A; Cutt S; Campbell B C; Maher F; Jakala P; Hartmann T; Beyreuther K; Masters C L; Li Q X. (Department of Pathology, The University of Melbourne, Parkville, Victoria, Australia.) AMERICAN JOURNAL OF PATHOLOGY, (1999 Oct) 155 (4) 1173-81. Journal code: 3RS; 0370502. ISSN: 0002-9440. Pub. country: United States. Language: English.

AB alpha-Synuclein (alphaSN), also termed the precursor of the non-Abeta component of **Alzheimer**'s disease (AD) amyloid (NACP), is a major component of Lewy bodies and Lewy neurites pathognomonic of **Parkinson**'s disease (PD) and dementia with Lewy bodies (DLB). A

fragment of alphaSN termed the non-Abeta component of AD amyloid (NAC) had
previously been identified as a constituent of AD amyloid plaques. To clarify the relationship of NAC and alphaSN with Abeta plaques, **antibodies** were raised to three domains of alphaSN. All **antibodies** produced punctate labeling of human cortex and strong labeling of Lewy bodies. Using **antibodies** to alphaSN(75-91) to label cortical and hippocampal sections of pathologically proven AD cases,
we found no evidence for NAC in Abeta amyloid plaques. Double labeling of **tissue** sections in mixed DLB/AD cases revealed alphaSN in dystrophic neuritic processes, some of which were in close association with Abeta plaques restricted to the CA1 hippocampal region. In brain homogenates alphaSN was predominantly recovered in the cytosolic fraction as a 16-kd protein on Western analysis; however, significant amounts of aggregated and alphaSN fragments were also found in urea extracts of **SDS**-insoluble material from DLB and PD cases. NAC **antibodies** identified an endogenous fragment of 6 kd in the cytosolic and urea-soluble brain fractions. This fragment may be produced as a consequence of alphaSN aggregation or alternatively may accelerate aggregation of the full-length alphaSN.

TI Non-Abeta component of **Alzheimer**'s disease amyloid (NAC) revisited. NAC and alpha-synuclein are not associated with Abeta amyloid.

AB alpha-Synuclein (alphaSN), also termed the precursor of the non-Abeta component of **Alzheimer**'s disease (AD) amyloid (NACP), is a major component of Lewy bodies and Lewy neurites pathognomonic of **Parkinson**'s disease (PD) and dementia with Lewy bodies (DLB). A fragment of alphaSN termed the non-Abeta component of AD amyloid (NAC) had
previously been identified as a constituent of AD amyloid plaques. To clarify the relationship of NAC and alphaSN with Abeta plaques, **antibodies** were raised to three domains of alphaSN. All **antibodies** produced punctate labeling of human cortex and strong labeling of Lewy bodies. Using **antibodies** to alphaSN(75-91) to label cortical and hippocampal sections of pathologically proven AD cases,
we found no evidence for NAC in Abeta amyloid plaques. Double labeling of **tissue** sections in mixed DLB/AD cases revealed alphaSN in dystrophic neuritic processes, some of which were in close association with Abeta plaques restricted to the CA1 hippocampal region. In brain homogenates alphaSN was predominantly recovered in the cytosolic fraction as a 16-kd protein on Western analysis; however, significant amounts of aggregated and alphaSN fragments were also found in urea extracts of **SDS**-insoluble material from DLB and PD cases. NAC **antibodies** identified an endogenous fragment of 6 kd in the cytosolic and urea-soluble brain fractions. This fragment may be produced as a consequence of alphaSN aggregation or alternatively may accelerate aggregation of the full-length alphaSN.

CT Check Tags: **Animal**; Human; Support, Non-U.S. Gov't
***Alzheimer Disease: ME, metabolism**
*Amyloid: ME, metabolism
Amyloid beta-Protein: ME, metabolism
Blotting, Western
Brain: ME, metabolism

Cells, Cultured
Embryo
Immunohistochemistry
Lewy Bodies: ME, metabolism
Microscopy, Fluorescence
***Nerve Tissue Proteins: ME, metabolism**
Neurofibrillary Tangles: ME, metabolism
Neurons: ME, metabolism
Rats
*Senile Plaques: ME, metabolism
Synaptophysin: ME, metabolism
tau Proteins: ME, metabolism
CN 0 (Amyloid); 0 (**Amyloid beta-Protein**); 0 (Nerve **Tissue** Proteins); 0 (Synaptophysin); 0 (non-Abeta component of AD amyloid protein); 0 (tau Proteins)

L107 ANSWER 4 OF 15 EMBASE COPYRIGHT 2001 ELSEVIER SCI. B.V.
2000004725 EMBASE Partial amino acid sequence of an **amyloid fibril protein** from unusual cutaneous cystic lesions in myeloma-associated **amyloidosis**. Akiyama T.; Seishima M.; Nojiri M.; Satoh M.; Ichiki Y.; Kitajima Y.. M. Seishima, Department of Dermatology, Gifu University School of Medicine, Gifu, Japan. seimarik@gumail.cc.gifu-u.ac.jp. European Journal of Dermatology 9/8 (624-628) 1999.
Refs: 25.
ISSN: 1167-1122. CODEN: EJDEE4. Pub. Country: France. Language: English. Summary Language: English.
AB Although common cutaneous lesions in myeloma-associated systemic **amyloidosis** are petechiae, purpura, ecchymoses, plaques, waxy, translucent or purpuric papules or nodules, we encountered an unusual case of myeloma- associated **amyloidosis** with multiple cystic nodules. We isolated amyloid substance from the cutaneous cystic nodules of this patient and characterized it ultrastructurally, immunologically, and biochemically. Electron **microscopy** demonstrated that amyloid substances isolated by distilled water were principally straight and non-branching fibrils with a diameter of 8 to 10 nm, which was morphologically similar to amyloid fibrils. **SDS**-PAGE showed that these fibrils consisted of the 20 kDa and 29 kDa peptides, which reacted with the **antibody** to kappa light chain of immunoglobulin by immunoblot study. Partial amino acid sequence of N-terminal residues of this 20 kDa peptide showed a homology to kappa immunoglobulin light chain of variable subgroup I. These results suggest that amyloid fibrils in this unusual case with cutaneous cystic nodules may be derived from kappa I light chain of immunoglobulin.
TI Partial amino acid sequence of an **amyloid fibril protein** from unusual cutaneous cystic lesions in myeloma-associated **amyloidosis**.
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patient and characterized it ultrastructurally, immunologically, and biochemically. Electron **microscopy** demonstrated that amyloid substances isolated by distilled water were principally straight and non-branching fibrils with a diameter of 8 to 10 nm, which was morphologically similar to amyloid fibrils. **SDS**-PAGE showed that these fibrils consisted of the 20 kDa and 29 kDa peptides, which reacted with the **antibody** to kappa light chain of immunoglobulin by immunoblot study. Partial amino acid sequence of N-terminal residues of this 20 kDa peptide showed a homology to kappa immunoglobulin light chain of variable subgroup I. These results suggest that amyloid fibrils in this unusual case with cutaneous cystic nodules may be derived from kappa I light chain of immunoglobulin.

CT Medical Descriptors:
***amyloidosis: DI, diagnosis**
*protein analysis
amino acid sequence
myeloma: ET, etiology
disease association
cell ultrastructure
histopathology
amino terminal sequence
protein purification
human
female
case report
human tissue
human cell
aged
article
Drug Descriptors:
*amyloid: EC, endogenous compound
***amyloid fibril protein**

L107 ANSWER 5 OF 15 MEDLINE DUPLICATE 2
1999227853 Document Number: 99227853. PubMed ID: 10211407.
Quantification of sub-femtomole amounts of **Alzheimer** amyloid beta peptides.
Potempska A; Mack K; Mehta P; Kim K S; Miller D L. (NYS Institute for Basic Research in Developmental Disabilities, Staten Island 10314, USA.)
AMYLOID, (1999 Mar) 6 (1) 14-21. Journal code: C2C; 9433802. ISSN: 1350-6129. Pub. country: United States. Language: English.

AB We evaluated methods for the quantitative Western blot analysis of A beta 1-40 and A beta 1-42. Both chromogenic and **chemiluminescent** detection methods gave similar sensitivities (0.15 fmol of A beta 1-40 and 0.3 fmol of A beta 1-42); however, the chromogenic method was more rapid, simpler, less expensive and gave fewer background problems; consequently, it yielded more reliable results. Adsorption to various types of laboratory plasticware can greatly interfere with the accurate measurement of A beta, but this can be prevented by the addition of **SDS** or bovine serum albumin. Among several methods for concentrating A beta from biological materials, immunoadsorption to Sepharose-bound **antibodies** was the most efficient. It yielded 50% recovery of 1 pM

A beta 1-42 or A beta 1-40 and so was a suitable method to measure A beta levels in human plasma. Through combined immunoadsorption and Western blotting we could determine the amounts of A beta isoforms secreted from 1
x 10(6) **cells** after a culture period as short as 1 h. This eliminates the need to use radiolabelling or over-expression to study A beta precursor processing Bovine serum contains subnanomolar A beta levels, similar to those that reportedly stimulate **cell** proliferation. That cultured **cells** quickly secrete these levels of A beta suggests that the peptide might exert an autocrine effect.

TI Quantification of sub-femtomole amounts of **Alzheimer** amyloid beta peptides.

AB We evaluated methods for the quantitative Western blot analysis of A beta 1-40 and A beta 1-42. Both chromogenic and **chemiluminescent** detection methods gave similar sensitivities (0.15 fmol of A beta 1-40 and
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CT Check Tags: **Animal**; Human; Support, Non-U.S. Gov't; Support, U.S. Gov't, P.H.S.

Alzheimer Disease: ME, metabolism
***Amyloid beta-Protein: AN, analysis**
Amyloid beta-Protein: IM, immunology
Antibody Specificity
*Blotting, Western: MT, methods
COS Cells
Culture Media
Enzyme-Linked Immunosorbent Assay
*Peptide Fragments: AN, analysis
Peptide Fragments: IM, immunology
Precipitin Tests
Sensitivity and Specificity
Tumor Cells, Cultured

CN 0 (**Amyloid beta-Protein**); 0 (Culture Media); 0 (Peptide Fragments); 0 (**amyloid beta-protein** (1-40)); 0 (beta-amyloid (1-42))

L107 ANSWER 6 OF 15 MEDLINE DUPLICATE 3

1998169534 Document Number: 98169534. PubMed ID: 9501253.
Alpha2-macroglobulin associates with beta-amyloid peptide and prevents fibril formation. Hughes S R; Khorkova O; Goyal S; Knaeblein J; Heroux J; Riedel N G; Sahasrabudhe S. (Biotechnology Group and the Central Nervous System Disease Group, Hoechst Marion Roussel, Inc., P.O. Box 6800, Bridgewater, NJ 08876-0800, USA.) PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, (1998 Mar 17) 95 (6) 3275-80. Journal code: PV3; 7505876. ISSN: 0027-8424. Pub. country: United States. Language: English.

AB We have used the **yeast** two-hybrid system to isolate cDNAs encoding proteins that specifically interact with the 42-aa beta-amyloid peptide (Abeta), a major constituent of senile plaques in **Alzheimer**'s disease. The carboxy terminus of alpha2-macroglobulin (alpha2M), a proteinase inhibitor released in response to inflammatory stimuli, was identified as a strong and specific interactor of Abeta, utilizing this system. Direct evidence for this interaction was obtained by co-immunoprecipitation of alpha2M with Abeta from the **yeast cell**, and by formation of **SDS**-resistant Abeta complexes in polyacrylamide gels by using synthetic Abeta and purified alpha2M. The association of Abeta with alpha2M and various purified amyloid binding proteins was assessed by employing a method measuring protein-protein interactions in liquid phase. The dissociation constant by this technique for the alpha2M-Abeta association using labeled purified proteins was measured (Kd = 350 nM). Electron **microscopy** showed that a 1:8 ratio of alpha2M to Abeta prevented fibril formation in solution; the same ratio to Abeta of another acute phase protein, alpha1-antichymotrypsin, was not active in preventing fibril formation in vitro. These results were corroborated by data obtained from an in vitro aggregation **assay** employing Thioflavine T. The interaction of alpha2M with Abeta suggests new pathway(s) for the clearance of the soluble amyloid peptide.

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CT Check Tags: Human
***Amyloid beta-Protein: ME, metabolism**
Biotinylation
DNA, Complementary
Hela Cells
Neurofibrils
*Peptide Fragments: ME, metabolism
Precipitin Tests
*Protease Inhibitors: ME, metabolism
Protein Binding
Thiazoles
alpha-Macroglobulins: GE, genetics
*alpha-Macroglobulins: ME, metabolism
CN 0 (**Amyloid beta-Protein**); 0 (DNA, Complementary); 0 (Peptide Fragments); 0 (Protease Inhibitors); 0 (Thiazoles); 0 (alpha-Macroglobulins); 0 (**amyloid beta -protein** (1-40)); 0 (beta-amyloid (1-42))

L107 ANSWER 7 OF 15 MEDLINE
97475986 Document Number: 97475986. PubMed ID: 9336237. Localization of perlecan (or a perlecan-related macromolecule) to isolated microglia in vitro and to microglia/macrophages following infusion of **beta-amyloid protein** into rodent hippocampus. Miller J D; Cummings J; Maresh G A; Walker D G; Castillo G M; Ngo C; Kimata K; Kinsella M G; Wight T N; Snow A D. (Department of Pathology, University of Washington, Seattle 98195-6480, USA.) GLIA, (1997 Oct) 21 (2) 228-43. Journal code: GLI; 8806785. ISSN: 0894-1491. Pub. country: United States. Language: English.
AB The origin of the heparan sulfate proteoglycan (PG), perlecan, in **beta-amyloid protein** (A beta)-containing amyloid deposits in **Alzheimer**'s disease (AD) brain is not known. In the present investigation we used indirect immunofluorescence, **SDS**-PAGE, and Western blotting with a specific perlecan core protein **antibody** to identify possible **cell** candidates of perlecan production in both primary **cell** cultures and in a rat infusion model. Double and triple-labeled indirect immunofluorescence was performed on dissociated primary rat septal cultures using **antibodies** for specific identification of **cell** types and for perlecan core protein. In mixed cultures of both embryonic day 18 (containing neurons and glia) and postnatal day 2-3 (devoid of neurons), microglia identified by labeling with OX-42 or anti-ED1 were the only **cell** type also double labeled with an affinity-purified polyclonal **antibody** against perlecan core protein. Similar immunolabeling of microglia with the anti-perlecan **antibody** was also observed in purified cultures of post-natal rat microglia. Analyses of PGs from cultured postnatal rat microglia by Western blotting using a polyclonal **antibody** against perlecan core protein revealed an approximately 400 kDa band in **cell** layer, which was intensified following heparitinase/heparinase digestion, suggestive of perlecan core protein. Other lower Mr bands were also found implicating either degradation of the 400 kDa core protein or the presence of separate and distinct gene products immunologically related to perlecan. Reverse transcription followed by polymerase chain reaction using human perlecan domain I

specific primers demonstrated perlecan mRNA in cultured human microglia derived from postmortem normal aged and AD brain. Following a 1-week continuous infusion of A beta (1-40) into rodent hippocampus, immunoperoxidase immunocytochemistry and double-labeled immunofluorescent studies revealed perlecan accumulation primarily localized to microglia/macrophages within the A beta infusion site. These studies have identified microglia/macrophages as one potential source of perlecan (or a perlecan-related macromolecule) which may be important for the ongoing accumulation of both perlecan and A beta in the amyloid deposits of AD.

TI Localization of perlecan (or a perlecan-related macromolecule) to isolated microglia in vitro and to microglia/macrophages following infusion of **beta-amyloid protein** into rodent hippocampus.

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CT Check Tags: **Animal**; Human; Male; Support, U.S. Gov't, P.H.S.
Amyloid beta-Protein: AD, administration & dosage
Amyloid beta-Protein: ME, metabolism
***Amyloid beta-Protein: PD, pharmacology**
Blotting, Western

Cells, Cultured
Electrophoresis, Polyacrylamide Gel
Fluorescent Antibody Technique
*Heparitin Sulfate: ME, metabolism
Hippocampus: DE, drug effects
*Hippocampus: ME, metabolism
Hippocampus: UL, ultrastructure
Immunoenzyme Techniques
Immunohistochemistry
*Macrophages: ME, metabolism
Macrophages: UL, ultrastructure
*Microglia: ME, metabolism
Microglia: UL, ultrastructure
Microinjections
Polymerase Chain Reaction
*Proteoglycans: ME, metabolism
RNA: BI, biosynthesis
RNA: IP, isolation & purification
Rats
Rats, Sprague-Dawley
CN 0 (**Amyloid beta-Protein**); 0 (Proteoglycans)

L107 ANSWER 8 OF 15 MEDLINE DUPLICATE 4
96067145 Document Number: 96067145. PubMed ID: 7589331. Clusterin (apoJ) alters the aggregation of amyloid beta-peptide (A beta 1-42) and forms slowly sedimenting A beta complexes that cause oxidative stress. Oda T; Wals P; Osterburg H H; Johnson S A; Pasinetti G M; Morgan T E; Rozovsky I;
Stine W B; Snyder S W; Holzman T F; +. (Neurogerontology Division, Andrus Gerontology Center, University of Southern California, Los Angeles 90089-0191, USA.) EXPERIMENTAL NEUROLOGY, (1995 Nov) 136 (1) 22-31. Journal code: EQF; 0370712. ISSN: 0014-4886. Pub. country: United States. Language: English.
AB Clusterin (apoJ), a multifunctional apolipoprotein made by **cells** in the brain and many other locations, is associated with aggregated amyloid beta-peptide (A beta) in senile and diffuse plaques of **Alzheimer**'s disease (AD). We observed that purified human serum clusterin partially blocked the aggregation of synthetic A beta 1-42, as shown by centrifugal **assays** (14,000g x 10 min) and by atomic force (scanning probe) **microscopy**. Slowly sedimenting A beta complexes were formed in the presence of clusterin, which included aggregates > 200 kDa that resist dissociation by low concentrations of **SDS**. Clusterin enhanced the oxidative stress caused by A beta, as **assayed** by oxidative stress in PC12 **cells** with MTT, which is widely used to estimate neurotoxicity. These indications of enhanced neurotoxicity by the MTT **assay** were observed in the highly aggregated rapidly sedimenting fraction, but also in more slowly sedimenting "soluble" forms. This novel activity of slowly sedimenting A beta may enhance the neurotoxicity of A beta deposits in AD brains, because soluble complexes have a potential for diffusing to damage distal neurons.
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CT Check Tags: **Animal**; Human; Support, Non-U.S. Gov't; Support, U.S. Gov't, P.H.S.
Alzheimer Disease: ME, metabolism
***Amyloid beta-Protein Precursor: ME, metabolism**
Complement Inactivators: ME, metabolism
*Complement Inactivators: PD, pharmacology
Dose-Response Relationship, Drug
Glycoproteins: IM, immunology
Glycoproteins: ME, metabolism
*Glycoproteins: PD, pharmacology
Immunohistochemistry
*Oxidative Stress
PC12 Cells: ME, metabolism
Radioligand Assay
Rats
Sucrose: PD, pharmacology

CN 0 (**Amyloid beta-Protein** Precursor); 0 (Complement Inactivators); 0 (Glycoproteins); 0 (clusterin)

L107 ANSWER 9 OF 15 MEDLINE DUPLICATE 5

93393542 Document Number: 93393542. PubMed ID: 8379923. High-level expression and in vitro mutagenesis of a fibrillogenic 109-amino-acid C-terminal fragment of **Alzheimer**'s-disease amyloid precursor protein. Gardella J E; Gorgone G A; Candela L; Ghiso J; Castano E M; Frangione B; Gorevic P D. (Department of Medicine, State University of New York, Stony Brook 11794.) BIOCHEMICAL JOURNAL, (1993 Sep 15) 294 (Pt 3) 667-74. Journal code: 9YO; 2984726R. ISSN: 0264-6021. Pub. country: ENGLAND: United Kingdom. Language: English.

AB We amplified DNA encoding the 3' 109 codons of **Alzheimer**'s-disease amyloid precursor protein (APP) inclusive of the beta protein (A beta) and cytoplasmic domains from cDNA using oligonucleotide primers designed to facilitate cloning into the T7 expression vector pT7Ad23K13. We also modified this construct to generate recombinant molecules incorporating two recently described APP mutants by site-directed mutagenesis. Both native C109 (deletion construct inclusive of the C-terminal 109 residues of APP) and constructs with a single mutation at codon 642 (T-->G, resulting in a substitution of glycine for valine) or a double mutation at codons 595 (G-->T, substituting asparagine for lysine) and 596 (A-->C, substituting leucine for methionine) were expressed in Escherichia coli to levels of 5-20% of total bacterial protein after

induction. The major constituent of expressed C109 protein had an apparent molecular mass of 16-18 kDa by **SDS**/PAGE and appeared to be the full-length construct by size and N-terminal microsequencing. Also present was a 4-5 kDa species that co-purified with C109, constituting only approximately 1% of expressed protein, which was revealed by Western-blot analysis with **antibodies** specific for A beta epitopes and after biotinylation of purified recombinant C109. This fragment shared N-terminal sequence with, and appeared to arise by proteolysis of, full-length C109 in biosynthetic labelling experiments. C109 spontaneously precipitated after dialysis against NaCl or water, and with prolonged (> 20 weeks) standing was found by electron **microscopy** to contain a minor (< 5%) fibrillar component that was reactive with **antibodies** to a C-terminal epitope of APP. Recombinant C109 appears to duplicate some of the biochemical and physicochemical properties of C-terminal A beta-inclusive fragments of APP that have been found in transfected **cells**, brain cortex and cerebral microvessels.

TI High-level expression and in vitro mutagenesis of a fibrillogenic 109-amino-acid C-terminal fragment of **Alzheimer**'s-disease amyloid precursor protein.

AB We amplified DNA encoding the 3' 109 codons of **Alzheimer**'s-disease amyloid precursor protein (APP) inclusive of the beta protein (A beta) and cytoplasmic domains from cDNA using oligonucleotide primers designed to facilitate cloning into the T7 expression vector pT7Ad23K13. We also modified this construct to generate recombinant molecules incorporating two recently described APP mutants by site-directed mutagenesis. Both native C109 (deletion construct inclusive of the C-terminal 109 residues of APP) and constructs with a single mutation at codon 642 (T-->G, resulting in a substitution of glycine for valine) or a double mutation at codons 595 (G-->T, substituting asparagine for lysine) and 596 (A-->C, substituting leucine for methionine) were expressed in Escherichia coli to levels of 5-20% of total bacterial protein after induction. The major constituent of expressed C109 protein had an apparent molecular mass of 16-18 kDa by **SDS**/PAGE and appeared to be the full-length construct by size and N-terminal microsequencing. Also present was a 4-5 kDa species that co-purified with C109, constituting only approximately 1% of expressed protein, which was revealed by Western-blot analysis with **antibodies** specific for A beta epitopes and after biotinylation of purified recombinant C109. This fragment shared N-terminal sequence with, and appeared to arise by proteolysis of, full-length C109 in biosynthetic labelling experiments. C109 spontaneously precipitated after dialysis against NaCl or water, and with prolonged (> 20 weeks) standing was found by electron **microscopy** to contain a minor (< 5%) fibrillar component that was reactive with **antibodies** to a C-terminal epitope of APP. Recombinant C109 appears to duplicate some of the biochemical and physicochemical properties of C-terminal A beta-inclusive fragments of APP that have been found in transfected **cells**, brain cortex and cerebral microvessels.

CT Check Tags: Human; Support, Non-U.S. Gov't; Support, U.S. Gov't, P.H.S.
Alzheimer Disease
Amino Acid Sequence
Amyloid: ME, metabolism
Amyloid beta-Protein Precursor: CH, chemistry
***Amyloid beta-Protein Precursor: GE, genetics**
Amyloid beta-Protein Precursor: IM, immunology
Base Sequence
Gene Expression
Macromolecular Systems
Molecular Sequence Data
Molecular Weight
Mutagenesis, Site-Directed
Oligodeoxyribonucleotides: CH, chemistry
Protein Binding
CN 0 (Amyloid); 0 (**Amyloid beta-Protein** Precursor); 0 (Macromolecular Systems); 0 (Oligodeoxyribonucleotides)

L107 ANSWER 10 OF 15 EMBASE COPYRIGHT 2001 ELSEVIER SCI. B.V.
93275958 EMBASE Document No.: 1993275958. Neurofibrillary tangles of Guamanian amyotrophic lateral sclerosis, parkinsonism-dementia and neurologically normal Guamanians contain a 4- to 4.5-kilodalton protein which is immunoreactive to anti-amyloid .beta./A4-protein **antibodies**. Guiroy D.C.; Mellini M.; Miyazaki M.; Hilbich C.; Safar J.; Garruto R.M.; Yanagihara R.; Beyreuther K.; Gajdusek D.C.. School of Medicine, Kyushu University,Fukuoka, Japan. Acta Neuropathologica 86/3 (265-274) 1993.
ISSN: 0001-6322. CODEN: ANPTAL. Pub. Country: Germany. Language: English. Summary Language: English.
AB Neurofibrillary tangles (NFT), one of the neurodegenerative features of **Alzheimer**'s disease, Down's syndrome and normal aging, is a constant, widespread neuropathological finding in Guamanian amyotrophic lateral sclerosis (ALS), parkinsonismdementia (PD) and in neurologically normal Guamanians, dying of causes other than ALS and PD. NFT in brain **tissue** sections of patients with Guamanian ALS and PD were immunoreactive to **antibodies** directed against a 43-amino acid synthetic peptide homologous to amyloid .beta./A4-protein (anti-SP43) associated with **Alzheimer**'s disease. NFT extracted from frozen brain **tissues** of Guamanian patients with ALS and PD and from **tissues** of neurologically normal Guamanians were congophilic and birefringent. By negative-stain electron **microscopy**, NFT preparations contained bundles and/or isolated single, straight, unpaired filaments in Guamanian ALS and occasionally pairing of filaments in neurologically normal Guamanians, measuring 5-20 nm in diameter. Formic acid digestion of NFT preparations, followed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and size-exclusion high-pressure liquid chromatography, showed a protein with an apparent molecular mass of 4- to 4.5-kDa, which by Western blot analysis was immunoreactive to anti-SP43. Immunoabsorption of purified NFT or SP43 with
anti-SP43 abolished immunostaining. Our study corroborate previous data that amyloid .beta./A4-protein is present in NFT in Guamanian PD. Furthermore, our data indicate that amyloid .beta./A4-protein is present in NFT in brain **tissues** of patients with Guamanian ALS and in neurologically normal Gumananians, suggesting a common mechanism of

amyloidogenesis with NFT formation in **Alzheimer**'s disease and normal brain aging.

TI Neurofibrillary tangles of Guamanian amyotrophic lateral sclerosis, parkinsonism-dementia and neurologically normal Guamanians contain a 4- to 4.5-kilodalton protein which is immunoreactive to anti-amyloid .beta./A4-protein **antibodies**.

AB Neurofibrillary tangles (NFT), one of the neurodegenerative features of **Alzheimer**'s disease, Down's syndrome and normal aging, is a constant, widespread neuropathological finding in Guamanian amyotrophic lateral sclerosis (ALS), parkinsonismdementia (PD) and in neurologically normal Guamanians, dying of causes other than ALS and PD. NFT in brain **tissue** sections of patients with Guamanian ALS and PD were immunoreactive to **antibodies** directed against a 43-amino acid synthetic peptide homologous to amyloid .beta./A4-protein (anti-SP43) associated with **Alzheimer**'s disease. NFT extracted from frozen brain **tissues** of Guamanian patients with ALS and PD and from **tissues** of neurologically normal Guamanians were congophilic and birefringent. By negative-stain electron **microscopy**, NFT preparations contained bundles and/or isolated single, straight, unpaired filaments in Guamanian ALS and occasionally pairing of filaments in neurologically normal Guamanians, measuring 5-20 nm in diameter. Formic acid digestion of NFT preparations, followed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and size-exclusion high-pressure liquid chromatography, showed a protein with an apparent molecular mass of 4- to 4.5-kDa, which by Western blot analysis was immunoreactive to anti-SP43. Immunoabsorption of purified NFT or SP43 with anti-SP43 abolished immunostaining. Our study corroborate previous data that amyloid .beta./A4-protein is present in NFT in Guamanian PD. Furthermore, our data indicate that amyloid .beta./A4-protein is present in NFT in brain **tissues** of patients with Guamanian ALS and in neurologically normal Gumananians, suggesting a common mechanism of amyloidogenesis with NFT formation in **Alzheimer**'s disease and normal brain aging.

CT Medical Descriptors:
*amyotrophic lateral sclerosis: ET, etiology
*immunoreactivity
*neurofibrillary tangle
***parkinson disease: ET, etiology**
adult
aged
aging
alzheimer disease: ET, etiology
article
birefringence
brain tissue
down syndrome: CN, congenital disorder
electron microscopy
female
gel permeation chromatography
human
human tissue
immunoadsorption
immunoblotting

immunohistochemistry
liquid chromatography
male
molecular weight
nerve degeneration: ET, etiology
neuropathology
polyacrylamide gel electrophoresis
priority journal
tissue
Drug Descriptors:
***amyloid beta protein**
antibody
brain protein: EC, endogenous compound
dodecyl sulfate sodium
protein: EC, endogenous compound
synthetic peptide
RN (**amyloid beta protein**) 109770-29-8; (dodecyl sulfate sodium) **151-21-3**; (protein) 67254-75-5

L107 ANSWER 11 OF 15 EMBASE COPYRIGHT 2001 ELSEVIER SCI. B.V.
93068505 EMBASE Document No.: 1993068505. Monoclonal **antibody** to .beta. peptide, recognizing amyloid deposits, neuronal **cells** and lipofuscin pigments in systemic organs. Takahashi H.; Utsuyama M.; Kurashima C.; Mori H.; Hirokawa K.. Brain Research Institute, University of Tokyo,Tokyo, Japan. Acta Neuropathologica 85/2 (159-166) 1993. ISSN: 0001-6322. CODEN: ANPTAL. Pub. Country: Germany. Language: English. Summary Language: English.

AB A monoclonal **antibody** (AmT-1) produced against synthetic amyloid .beta. peptide (1-28 residues) was revealed to be reactive with amyloid .beta. peptide blotted on nitrocellulose membrane, but not with that dissolved in sodium dodecyl sulfate and electrophoresed. AmT-1 immunostained senile plaques of typical, primitive and diffuse type, as well as amyloid deposits in cerebral vessels. It also reacted with neuronal and glial **cells** of normal and **Alzheimer**'s disease (AD) brains. In addition, AmT-1 was also reactive strongly with lipofuscin pigments of adrenal reticular **cells**, and weakly with those of eccrine glands and liver **cells**. A rat neural **cell** line (PC12h) was reactive with AmT-1. By immunoelectron **microscopy**, a positive reaction was seen in ribosomes along the rough endoplasmic reticulum of nerve **cells** and PC12h **cells**. By immunoprecipitation, AmT1 reacted with a band at 36 kDa in the brain homogenates from Ad patients as well as from normal aged subjects. By immunoblotting analysis, AmT1 reacted with a band at 36 kDa in the cytosolic fraction of PC12 **cells**, and three bands (12-17 kDa) in the lipopigment fraction of the adrenal gland. These findings suggest that the cerebral amyloid deposits contain substance(s) having an epitope common to neuronal **cells** and lipofuscin pigments. The possible relationship between cerebral amyloid deposits and lipofuscin pigments in systemic organs is discussed.

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CT Medical Descriptors:
***nerve cell**
*senile plaque: ET, etiology
adrenal cell
aged
alzheimer disease: ET, etiology
animal cell
article
brain blood vessel
brain homogenate
clinical article
controlled study
glia cell
human
human tissue
immunoblotting
immunoelectron microscopy
immunoprecipitation
liver cell
nerve cell culture
nonhuman
priority journal
rat
ribosome
rough endoplasmic reticulum
sweat gland
Drug Descriptors:
***amyloid beta protein: EC, endogenous compound**
*lipofuscin: EC, endogenous compound
amyloid: EC, endogenous compound
dodecyl sulfate sodium
monoclonal antibody
pyroxylin

RN (**amyloid beta protein**) 109770-29-8; (amyloid) 11061-24-8; (dodecyl sulfate sodium) **151-21-3**; (pyroxylin) 9004-70-0

L107 ANSWER 12 OF 15 MEDLINE
93161132 Document Number: 93161132. PubMed ID: 8431789. Synthetic **Alzheimer** amyloid beta/A4 peptides enhance production of complement C3 component by cultured microglial **cells**. Haga S; Ikeda K; Sato M; Ishii T. (Department of Ultrastructure and Histochemistry, Tokyo Institute of Psychiatry, Japan.) BRAIN RESEARCH, (1993 Jan 22) 601 (1-2) 88-94. Journal code: B5L; 0045503. ISSN: 0006-8993. Pub. country: Netherlands. Language: English.

AB Primary microglial cultures prepared from newborn mice showed the production and release of the third component of complement (C3). Newly synthesized [35S]methionine-labelled C3 was purified by immunoprecipitation using anti-C3-**antibody**. C3 was detected by **SDS**-PAGE and fluoroaraphy of the immunoprecipitated protein from **cell** lysates as a 195 kDa band, and from the supernatants of cultures as two major bands corresponding to the C3 alpha-chain (125 kDa) and beta-chain (75 kDa), consistent with known C3 characteristics. Increased biosynthesis of C3 was elicited by endotoxin lipopolysaccharide (LPS). Further, the synthesis of C3 was increased 5-10-fold in response to various synthetic peptides corresponding to the amyloid beta/A4 protein, which is the main constituent of extracellular amyloid deposits in **Alzheimer**'s disease (AD). The increased synthesis of C3 was shown to be dose dependent at concentrations of beta/A4 peptide ranging from 10 micrograms/ml to 50 micrograms/ml. These results suggest that complement components found previously in amyloid deposits may be partly derived from reactive microglia preferentially associated with senile plaques in AD brain.

TI Synthetic **Alzheimer** amyloid beta/A4 peptides enhance production of complement C3 component by cultured microglial **cells**.

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CT Check Tags: **Animal**
***Amyloid beta-Protein: PD, pharmacology**
Astrocytes: DE, drug effects
Astrocytes: ME, metabolism

Cells, Cultured
*Complement 3: BI, biosynthesis
Electrophoresis, Polyacrylamide Gel
Fluorescent Antibody Technique
Granulocyte-Macrophage Colony-Stimulating Factor: PD, pharmacology
Indicators and Reagents
Lipopolysaccharides: PD, pharmacology
Macrophages: DE, drug effects
Macrophages: ME, metabolism
Mice
Mice, Inbred BALB C
Neuroglia: DE, drug effects
*Neuroglia: ME, metabolism
Parathyroid Hormones: PD, pharmacology
Phagocytosis: DE, drug effects
Precipitin Tests
Stimulation, Chemical
CN 0 (**Amyloid beta-Protein**); 0 (Complement 3); 0 (Indicators and Reagents); 0 (Lipopolysaccharides); 0 (Parathyroid Hormones)

L107 ANSWER 13 OF 15 MEDLINE
92366476 Document Number: 92366476. PubMed ID: 1502155. Intracellular accumulation and resistance to degradation of the **Alzheimer** amyloid A4/beta protein. Knauer M F; Soreghan B; Burdick D; Kosmoski J; Glabe C G. (Department of Molecular Biology and Biochemistry, University of California, Irvine 92717.) PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, (1992 Aug 15) 89 (16) 7437-41. Journal code: PV3; 7505876. ISSN: 0027-8424. Pub. country: United States. Language: English.
AB The A4 or beta protein is a peptide that constitutes the major protein component of senile plaques in **Alzheimer** disease. The A4/beta protein is derived from a larger, transmembrane amyloid precursor protein (APP). The putative abnormal processing events leading to amyloid accumulation are largely unknown. Here we report that a 42-residue synthetic peptide, beta 1-42, corresponding to one of the longer forms of the A4/beta protein, accumulates in cultured human skin fibroblasts and is stable for at least 3 days. The peptide appears to accumulate intracellularly, since it does not accumulate under conditions that prevent endocytosis and accumulation is correlated with the acquisition of resistance to removal by trypsin digestion. This intracellular accumulation is also correlated with the ability of the peptide to aggregate as determined by **SDS**/polyacrylamide gel electrophoresis. At low concentrations of the beta 1-42 peptide, which favor the nonaggregated state, no accumulation is observed. Shorter peptide analogs (28 or 39 residues) that are truncated at the C terminus, which lack the ability to aggregate in **SDS** gels, fail to accumulate. The accumulated intracellular beta 1-42 peptide is in an aggregated state and is contained in a dense organellar compartment that overlaps the distribution of late endosomes or secondary lysosomes. Immunofluorescence of the internalized peptide in permeabilized **cells** reveals that it is contained in granular deposits, consistent with localization in late endosomes or secondary lysosomes.

Sequence analysis indicates that some of the internalized peptide is subject to N-terminal trimming. These results suggest that the aggregated A4/beta protein may be resistant to degradation and suggest that the A4/beta protein may arise, at least in part, by endosomal or lysosomal processing of APP. Our results also suggest that relatively nonspecific proteolysis may be sufficient to generate the A4/beta protein if this part of APP is selectively resistant to proteolysis.

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CT Check Tags: Human; Support, U.S. Gov't, P.H.S.
Amino Acid Sequence
***Amyloid beta-Protein: ME, metabolism**
Biological Transport
Cells, Cultured
Fluorescent Antibody Technique
Infant, Newborn
Molecular Sequence Data
Peptide Fragments: ME, metabolism
Peptides: CS, chemical synthesis
Peptides: ME, metabolism
Skin: ME, metabolism

CN 0 (**Amyloid beta-Protein**); 0 (Peptide Fragments); 0 (Peptides)

L107 ANSWER 14 OF 15 MEDLINE

89079776 Document Number: 89079776. PubMed ID: 3060472. Isolation and chemical characterization of **Alzheimer**'s disease paired helical filament cytoskeletons: differentiation from amyloid plaque core protein. Roher A E; Palmer K C; Chau V; Ball M J. (Department of Anatomy, Wayne State University School of Medicine, Detroit, Michigan 48201.) JOURNAL OF CELL BIOLOGY, (1988 Dec) 107 (6 Pt 2) 2703-16. Journal code: HMV; 0375356. ISSN: 0021-9525. Pub. country: United States. Language: English.

AB The paired helical filaments (PHFs) of **Alzheimer**'s disease were purified by a strategy in which the neurons and amyloid plaque cores of protein (APCP) were initially isolated. This was achieved by several steps of isocratic sucrose centrifugations of increasing molarity and a discontinuous isotonic Percoll density gradient. After collagenase elimination of contaminating blood vessels, lysis of neurons was produced by **SDS** treatment. The released PHF cytoskeletons were separated from contaminating APCP and lipofuscin by sucrose density gradient. A final step consisted in the chemical purification of highly enriched PHFs and APCP components via a formic acid to guanidine hydrochloride transition. PHFs and APCPs were fractionated by size exclusion HPLC and further characterized and quantitated by automatic amino acid analysis. We also present some of the morphological and immunochemical characteristics of PHF **polypeptides** and APCP. Our studies indicate that apart from differences in localization and morphology, PHF and APCP significantly differ in (a) chemical structure (peptide and amino acid composition); (b) epitope specificity (antiubiquitin, antitau, antineurofilament); (c) physicochemical properties (structural conformation in guanidine hydrochloride); and (d) thioflavine T **fluorescence** emission. These parameters strongly suggest important differences in the composition and, probably, in the etiopathology of PHF and APCP of **Alzheimer**'s disease.

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CT Check Tags: Human; Support, Non-U.S. Gov't; Support, U.S. Gov't, P.H.S.
***Alzheimer Disease: PA, pathology**
*Amyloid: AN, analysis
Amyloid: IP, isolation & purification
Amyloid beta-Protein
*Brain: PA, pathology
Centrifugation, Density Gradient
Chromatography, High Pressure Liquid
*Cytoskeleton: AN, analysis
Cytoskeleton: UL, ultrastructure
Fluorescent Antibody Technique
Immunohistochemistry
Microscopy, Electron
Nerve Tissue Proteins: AN, analysis
*Neurons: AN, analysis
Neurons: UL, ultrastructure

CN 0 (Amyloid); 0 (**Amyloid beta-Protein**); 0 (Nerve **Tissue** Proteins)

L107 ANSWER 15 OF 15 MEDLINE

89065113 Document Number: 89065113. PubMed ID: 2904381. The amyloid percursor protein of **Alzheimer** disease is expressed as a 130 kDa **polypeptide** in various cultured **cell** types.
Autilio-Gambetti L; Morandi A; Tabaton M; Schaetzle B; Kovacs D; Perry G; Sharma S; Cornette J; Greenberg B; Gambetti P. (Division of Neuropathology, Case Western Reserve University, Cleveland, OH 44106.)
FEBS LETTERS, (1988 Dec 5) 241 (1-2) 94-8. Journal code: EUH; 0155157. ISSN: 0014-5793. Pub. country: Netherlands. Language: English.

AB The vascular and parenchymal amyloid deposits in **Alzheimer** disease (AD), normal aging and Down syndrome are mainly composed of a 4 kDa **polypeptide** (A4), which derives from a larger precursor protein (APP). There is evidence that APP is a transmembrane glycoprotein present in most **tissues**, but the characteristics of APP in intact **cells** are not yet known. In order to investigate this issue, we examined the immunoreactivity of fibroblasts of human and nonhuman **cell** lines with antisera raised to synthetic peptides corresponding to A4 and to two other domains of the APP. All three antisera recognized a 130 kDa **polypeptide** (APP-130) in immunoblots from all **cell** lines. In fibroblasts, an additional **polypeptide** of 228 kDa (APP-228) was recognized by the antiserum to A4. In immunoblots of two dimensional gels, APP-130 showed a pI of 6.2, while APP-228 failed to focus in the pH range of 4.7-7.0. Sequential extractions of **cells** with buffer and with **Triton X-100** indicate that APP-130 is extractable with nonionic detergents at high ionic strength, whereas 228 kDa APP is a cystolic

component. Immunofluorescence staining is consistent with an intracellular perinuclear and plasma membrane localization. It is concluded that APP-130 and APP-228 are two forms of the APP which result from extensive posttranslational modifications of a smaller original gene product. It is likely that APP undergoes similar posttranslational modifications in different **cell** types.

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CT Check Tags: Human; Support, U.S. Gov't, P.H.S.
***Alzheimer Disease: ME, metabolism**
*Amyloid: BI, biosynthesis
Amyloid beta-Protein Precursor
Cells, Cultured
Electrophoresis, Polyacrylamide Gel
Fibroblasts: ME, metabolism
Fluorescent Antibody Technique
Immune Sera
Immunoblotting
*Protein Precursors: BI, biosynthesis

CN 0 (Amyloid); 0 (**Amyloid beta-Protein** Precursor); 0 (Immune Sera); 0 (Protein Precursors)

L106 ANSWER 1 OF 1 CAPLUS COPYRIGHT 2001 ACS

2001:371235 Document No. 135:105944 Accumulation of mutant huntingtin fragments in aggresome-like inclusion bodies as a result of insufficient protein degradation. Waelter, Stephanie; Boeddrich, Annett; Lurz, Rudi; **Scherzinger, Eberhard**; Lueder, Gerhild; **Lehrach, Hans**; Wanker, Erich E. (Max-Planck-Institut fur Molekulare Genetik, Berlin, D-14195, Germany). Mol. Biol. Cell, 12(5), 1393-1407 (English) 2001. CODEN: MBCEEV. ISSN: 1059-1524. Publisher: American Society for Cell Biology.

AB The huntingtin exon 1 proteins with a polyglutamine repeat in the pathol. range (51 or 83 glutamines), but not with a polyglutamine tract in the normal range (20 glutamines), form aggresome-like perinuclear inclusions in human 293 Tet-Off cells. These structures contain aggregated, ubiquitinated huntingtin exon 1 protein with a characteristic fibrillar morphol. Inclusion bodies with truncated huntingtin protein are formed at centrosomes and are surrounded by vimentin filaments. Inhibition of proteasome activity resulted in a twofold increase in the amt. of ubiquitinated, SDS-resistant aggregates, indicating that inclusion bodies accumulate when the capacity of the ubiquitin-proteasome system to degrade aggregation-prone huntingtin protein is exhausted. Immunofluorescence and electron microscopy with immunogold labeling revealed that the 20S, 19S, and 11S subunits of the 26S proteasome, the mol. chaperones BiP/GRP78, Hsp70, and Hsp40, as well as the RNA-binding protein TIA-1, the potential chaperone 14-3-3, and .alpha.-synuclein colocalize with the perinuclear inclusions. In 293 Tet-Off cells, inclusion body formation also resulted in cell toxicity and dramatic ultrastructural changes such as indentations and disruption of the nuclear envelope. Concn. of mitochondria around the inclusions and cytoplasmic vacuolation were also obsd. Together these findings support the hypothesis that the ATP-dependent ubiquitin-proteasome system is a potential target for therapeutic interventions in glutamine repeat disorders.

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